

## Complete Listing of Claims

Claims 1-111. Cancelled

Claim 112. (Currently amended). An isolated or purified cell which is recombinant or genetically modified to contain [or] and co-express

i) a cytidine monophosphate sialic acid (CMP- SA)-synthase gene wherein said CMP- SA synthase gene encodes a polypeptide that catalyzes the formation of CMP-SA from SA and cytidine triphosphate (CTP) encoding the, and wherein said polypeptide is

a) a polypeptide represented by SEQ ID NO:4 or

b) a variant polypeptide thereof that contains conservative amino acid substitutions and in which CMP-SA-synthase structure and function are conserved, and

ii) a sialic acid phosphate synthase (SAS) gene wherein said SAS gene encodes a polypeptide that catalyzes the formation of sialic acid phosphate from N-acetylmannosamine (ManNAc) and phosphoenolpyruvate (PEP), and encoding: and wherein said polypeptide is

i a) [the] a polypeptide represented by SEQ ID NO: 6 or a variant polypeptide thereof that contains conservative amino acid substitutions and in which SAS structure and function are conserved,, or

ii b) ) [the] a polypeptide represented by SEQ ID NO: 8 or a functional variant polypeptide thereof that contains conservative amino acid substitutions and in which SAS structure and function are conserved, and

wherein said cell is capable of producing donor substrate CMP-SA above levels produced before said cell was made recombinant or genetically modified, when provided with N-acetylmannosamine ( ManNAc ).

Claim 113. (Previously presented) The isolated or purified cell of claim 112, which is an insect cell.

Claim 114. (Previously presented) The insect cell of claim 113, wherein said insect cell is of a species selected from the group consisting of:

- (a) *Spodoptera frugiperda*;
- (b) *Trichoplusia ni*;
- (c) *Estigmena acrea*; and,
- (d) *Drosophila*.

Claim 115. (Previously presented) The isolated or purified cell of claim 112, which is a yeast cell.

Claim 116. (Previously presented) The isolated or purified cell of claim 112, which is a plant cell.

Claim 117. (Previously presented) The isolated or purified cell of claim 112, which is a bacterial cell.

Claim 118. (Previously presented) The isolated or purified cell of claim 112, which is a fungal cell.

Claim 119. (Previously presented) The isolated or purified cell of claim 112, wherein said cell is a mammalian cell.

Claim 120. (Previously presented) The isolated or purified cell of claim 112, wherein the donor substrate CMP-SA is CMP-Neu5Ac (cytidine monophosphate-*N*-acetylneuraminic acid).

Claim 121. (Previously presented) The isolated or purified cell of claim 112 wherein said cell is provided with ManNAc by addition of ManNAc to media in which said cell is grown.

Claim 122. (Previously presented) The isolated or purified cell of claim 112 wherein said cell is provided with ManNAc by genetically engineering said cell to catalyze synthesis of ManNAc.

Claim 123. (Previously presented) The isolated or purified cell of claim 122, wherein said cell is genetically engineered to contain or express an epimerase that synthesizes ManNAc.

Claim 124. (Previously presented) The isolated or purified cell of claim 123, wherein said epimerase is UDP-GlcNAc-2 epimerase.

Claim 125. (Previously presented) The isolated or purified cell of claim 123, wherein said epimerase is GlcNAc-2 epimerase.

Claim 126. (Previously presented) The isolated or purified cell of claim 112, wherein said CMP-sialic acid synthase gene and said sialic acid phosphate synthase gene are isolated from a human source.

Claim 127. (Currently amended). An isolated or purified cell which is recombinant or genetically modified to contain or co-express

i) a ~~CMP~~ cytidine monophosphate-sialic acid (CMP-SA) ~~acid~~ synthase gene, wherein said CMP-SA-synthase gene encodes a polypeptide that catalyzes the formation of CMP-SA from SA and cytidine triphosphate (CTP), and wherein said CMP-SA synthase gene comprises

a) a nucleotide sequence represented by SEQ ID NO: 3, or

b) a polynucleotide that hybridizes to the nucleotide sequence represented by SEQ ID NO:3 under stringent conditions, and

ii) a sialic acid phosphate synthase (SAS) gene wherein said SAS gene encodes a polypeptide that catalyzes the formation of sialic acid phosphate from N-acetylmannosamine (ManNAc) and phosphoenolpyruvate (PEP), and wherein said SAS gene comprises

a) a nucleotide sequence represented by SEQ ID NO: 5, or

b) a polynucleotide that hybridizes to the nucleotide sequence represented by SEQ ID NO:5 under stringent conditions,

and wherein said cell is capable of producing donor substrate CMP-SA above levels

produced before said cell was made recombinant or genetically modified, when provided with ~~N~~-acetylmannosamine (ManNAc).

Claim 128. (Previously presented) The isolated or purified cell of claim 127, which is an insect cell.

Claim 129. (Previously presented) The insect cell of claim 128, wherein said insect cell is of a species selected from the group consisting of:

- (a) *Spodoptera frugiperda*;
- (b) *Trichoplusia ni*;
- (c) *Estigmena acrea*; and,
- (d) *Drosophila*.

Claim 130. (Previously presented) The isolated or purified cell of claim 127, which is a yeast cell.

Claim 131. (Previously presented) The isolated or purified cell of claim 127, which is a plant cell.

Claim 132. (Previously presented) The isolated or purified cell of claim 127, which is a bacterial cell.

Claim 133. (Previously presented) The isolated or purified cell of claim 127, which is a fungal cell.

Claim 134. (Previously presented) The isolated or purified cell of claim 127, wherein said cell is a mammalian cell.

Claim 135. (Previously presented) The isolated or purified cell of claim 127, wherein the donor substrate CMP-SA is CMP-Neu5Ac (cytidine monophosphate-N-acetylneuraminic acid).

Claim 136. (Previously presented) The isolated or purified cell of claim 127 wherein said cell is provided with ManNAc by addition of ManNAc to media in which said cell is grown.

Claim 137. (Previously presented) The isolated or purified cell of claim 127 wherein said cell is provided with ManNAc by genetically engineering said cell to catalyze synthesis of ManNAc.

Claim 138. (Previously presented) The isolated or purified cell of claim 137, wherein said cell is genetically engineered to contain or express an epimerase that synthesizes ManNAc.

Claim 139. (Previously presented) The isolated or purified cell of claim 138, wherein said epimerase is UDP-GlcNAc-2 epimerase.

Claim 140. (Previously presented) The isolated or purified cell of claim 138, wherein said epimerase is GlcNAc-2 epimerase.

Claim 141. (Previously presented) The isolated or purified cell of claim 127, wherein said CMP-sialic acid synthase gene and said sialic acid phosphate synthase gene are isolated from a human source.

Claims 142-150. Cancelled

Claim 151. (Currently amended) An isolated or purified cell that is recombinant or genetically modified to contain and co-express

i) a ~~CMP~~ cytidine monophosphate-sialic acid (CMP-SA) synthase gene, wherein said [( ]CMP-SA[ )] synthase gene encodes a polypeptide that catalyzes the formation of CMP-SA from SA and cytidine triphosphate (CTP), and wherein said polypeptide is

a) [the] a polypeptide represented by SEQ ID NO:4 or

b) a variant of the polypeptide represented by SEQ ID NO:4 that contains

conservative amino acid substitutions and in which CMP-SA structure and function are conserved; and

ii) a sialic acid phosphate synthase (SAS) gene, wherein said SAS gene encodes a polypeptide that catalyzes the formation of sialic acid phosphate from N-acetylmannosamine (ManNAc) and phosphoenolpyruvate (PEP), and said (SAS) gene encoding: wherein said polypeptide is

a) [the] a polypeptide represented by SEQ ID NO: 6; or

b) a variant of the polypeptide represented by SEQ ID NO:6 that contains conservative amino acid substitutions and in which SAS structure and function are conserved; or

c) [the] a polypeptide represented by SEQ ID NO: 8; or

d) a variant of the polypeptide represented by SEQ ID NO: 8 that contains conservative amino acid substitutions and in which SAS structure and function are conserved,

said cell producing the donor substrate CMP-SA above a level produced before said cell was made recombinant or genetically modified, wherein the donor substrate CMP-SA is CMP-KDN (cytidine monophosphate-2-keto-3-deoxy-D-glycero-D-galacto-nonoic acid).

Claim 152. (Previously presented) The isolated or purified cell of claim 151, which is an insect cell.

Claim 153. (Previously presented) The insect cell of claim 152, wherein said insect cell is of a species selected from the group consisting of:

(a) *Spodoptera frugiperda*;

(b) *Trichoplusia ni*;

(c) *Estigmene acrea*; and,

(d) *Drosophila*.

Claim 154. (Previously presented) The isolated or purified cell of claim 151, which is a yeast cell.

Claim 155. (Previously presented) The isolated or purified cell of claim 151, which is a plant cell.

Claim 156. (Previously presented) The isolated or purified cell of claim 151, which is a bacterial cell.

Claim 157. (Previously presented) The isolated or purified cell of claim 151, which is a fungal cell.

Claim 158. (Previously presented) The isolated or purified cell of claim 151, which is a mammalian cell.

Claim 159. (Previously presented) The isolated or purified cell of claim 151, wherein said CMP-sialic acid synthase gene and said sialic acid phosphate synthase gene are isolated from a human source.

Claim 160. (Currently amended) An isolated or purified cell that is recombinant or genetically modified to contain and co-express

i) a ~~CMP~~ cytidine monophosphate-sialic acid (CMP-SA) synthase gene, wherein said CMP-SA-synthase gene encodes a polypeptide that catalyzes the formation of CMP-SA from SA and cytidine triphosphate (CTP), and wherein said CMP- ~~sialic acid~~ SA synthase gene comprises

a) a nucleotide sequence represented by SEQ ID NO: 3, or

b) a polynucleotide that hybridizes to the nucleotide sequence represented by SEQ ID NO:3 under stringent conditions, and

ii) a sialic acid phosphate synthase gene wherein said SAS gene encodes a polypeptide that catalyzes the formation of sialic acid phosphate from N-acetylmannosamine (ManNAc) and phosphoenolpyruvate (PEP), and wherein said ~~sialic acid phosphate synthase~~ SAS gene comprises

a) a nucleotide sequence represented by SEQ ID NO: 5, or

b) a polynucleotide that hybridizes to the nucleotide sequence represented by SEQ ID NO:5 under stringent conditions,

said cell producing the donor substrate CMP-SA above a level produced before said cell was made recombinant or genetically modified, wherein the donor substrate CMP-SA is CMP-KDN (cytidine monophosphate-2-keto-3-deoxy-D-*glycero*-D-*galacto*-nonoic acid).

Claim 161. (Previously presented) The isolated or purified cell of claim 160, which is an insect cell.

Claim 162. (Previously presented) The insect cell of claim 161, wherein said insect cell is of a species selected from the group consisting of:

(a) *Spodoptera frugiperda*;

(b) *Trichoplusia ni*;

(c) *Estigmena acrea*; and,

(d) *Drosophila*.

Claim 163. (Previously presented) The isolated or purified cell of claim 160, which is a yeast cell.

Claim 164. (Previously presented) The isolated or purified cell of claim 160, which is a plant cell.

Claim 165. (Previously presented) The isolated or purified cell of claim 160, which is a bacterial cell.

Claim 166. (Previously presented) The isolated or purified cell of claim 160, which is a fungal cell.

Claim 167. (Previously presented) The isolated or purified cell of claim 160, which is a



mammalian cell.

Claim 168. (Previously presented) The isolated or purified cell of claim 160, wherein said CMP-sialic acid synthase gene and said sialic acid phosphate synthase gene are isolated from a human source.

Claims 169-184. Cancelled

Claim 185. (Currently amended) An isolated or purified cell from a recombinant or genetically engineered cell line which contains and co-expresses

i) a ~~CMP~~ cytidine monophosphate-sialic acid (CMP-SA) synthase gene, wherein said CMP-SA-synthase gene encodes a polypeptide that catalyzes the formation of CMP-SA from SA and cytidine triphosphate (CTP), and wherein said (CMP-SA) synthase gene encodes polypeptide is

a) ~~the~~ a polypeptide represented by SEQ ID NO:4 or

b) a variant of the polypeptide represented by SEQ ID NO:4 that contains conservative amino acid substitutions and in which CMP-SA structure and function are conserved; and

ii) a sialic acid phosphate synthase (SAS) gene, wherein said SAS gene encodes a polypeptide that catalyzes the formation of sialic acid phosphate from N-acetylmannosamine (ManNAc) and phosphoenolpyruvate (PEP), and said (SAS) gene encoding: wherein said polypeptide is

a) ~~the~~ a polypeptide represented by SEQ ID NO: 6; or

b) a variant of the polypeptide represented by SEQ ID NO:6 that contains conservative amino acid substitutions and in which SAS structure and function are conserved; or

c) ~~the~~ a polypeptide represented by SEQ ID NO: 8; or

d) a variant of the polypeptide represented by SEQ ID NO: 8 that contains

conservative amino acid substitutions and in which SAS structure and function are conserved,

to produce said cell producing a donor substrate CMP-SA at a higher level than a cell from a parent cell line corresponding to said recombinant or genetically engineered cell line, when provided with N-acetylmannosamine (ManNAc).

Claim 186. (Previously presented) The isolated or purified cell of claim 185, which is an insect cell.

Claim 187. (Previously presented) The insect cell of claim 186, wherein said insect cell is of a species selected from the group consisting of:

- (a) *Spodoptera frugiperda*;
- (b) *Trichoplusia ni*;
- (c) *Estigmena acrea*; and,
- (d) *Drosophila*.

Claim 188. (Previously presented) The isolated or purified cell of claim 185, which is a yeast cell.

Claim 189. (Previously presented) The isolated or purified cell of claim 185, which is a plant cell.

Claim 190. (Previously presented) The isolated or purified cell of claim 185, which is a bacterial cell.

Claim 191. (Previously presented) The isolated or purified cell of claim 185, which is a fungal cell.

Claim 192. (Previously presented) The isolated or purified cell of claim 185, which is a mammalian cell.

Claim 193. (Previously presented) The isolated or purified cell of claim 185, wherein the donor substrate CMP-SA is CMP-Neu5Ac (cytidine monophosphate-*N*-acetylneuraminic acid).

Claim 194. (Previously presented) The isolated or purified cell of claim 185 wherein said CMP-sialic acid synthase gene and said sialic acid phosphate synthase gene are isolated from a human source.

Claim 195. (Previously presented) The cell of claim 185 wherein said cell is provided with ManNAc by addition of ManNAc to media in which said cell is grown.

Claim 196. (Previously presented) The cell of claim 185 wherein said cell is provided with ManNAc by genetically engineering said cell to catalyze synthesis of ManNAc.

Claim 197. (Previously presented) The cell of claim 196, wherein said cell is genetically engineered to contain or express an epimerase that synthesizes ManNAc.

Claim 198. (Previously presented) The cell of claim 197, wherein said epimerase is UDP-GlcNAc-2 epimerase.

Claim 199. (Previously presented) The cell of claim 197, wherein said epimerase is GlcNAc-2 epimerase.

Claim 200. (Previously presented) The cell of claim 185 wherein said cell also contains SA precursor GlcNAc above endogenous levels as a result of supplementation of cell growth medium with SA precursor GlcNAc.

Claim 201. (Currently amended) An isolated or purified cell from a recombinant or genetically engineered cell line which contains and co-expresses

i) a CMP cytidine monophosphate -sialic acid (CMP-SA) - synthase gene, wherein said CMP- SA-synthase gene encodes a polypeptide that catalyzes the formation of CMP-SA from SA and cytidine triphosphate (CTP), and wherein said CMP- sialic acid SA synthase gene comprises

a) a nucleotide sequence represented by SEQ ID NO: 3, or

b) a polynucleotide that hybridizes to the nucleotide sequence represented by SEQ ID NO:3 under stringent conditions, and

ii) a sialic acid phosphate synthase gene wherein said SAS gene encodes a polypeptide that catalyzes the formation of sialic acid phosphate from N-acetylmannosamine (ManNAc) and phosphoenolpyruvate (PEP), and wherein said sialic acid phosphate synthase gene comprises

a) a nucleotide sequence represented by SEQ ID NO: 5, or

b) a polynucleotide that hybridizes to the nucleotide sequence represented by SEQ ID NO:5 under stringent conditions,

to produce said cell producing a donor substrate CMP-SA at a higher level than a cell from a parent cell line corresponding to said recombinant or genetically engineered cell line, when provided with N-acetylmannosamine ( ManNAc ).

Claim 202. (Previously presented) The isolated or purified cell of claim 201, which is an insect cell.

Claim 203. (Previously presented) The insect cell of claim 202, wherein said insect cell is of a species selected from the group consisting of:

(a) *Spodoptera frugiperda*;

(b) *Trichoplusia ni*;

(c) *Estigmena acrea*; and,

(d) *Drosophila*.

Claim 204. (Previously presented) The isolated or purified cell of claim 201, which is a yeast cell.

Claim 205. (Previously presented) The isolated or purified cell of claim 201, which is a plant cell.

Claim 206. (Previously presented) The isolated or purified cell of claim 201, which is a bacterial cell.

Claim 207. (Previously presented) The isolated or purified cell of claim 201, which is a fungal cell.

Claim 208. (Previously presented) The isolated or purified cell of claim 201, which is a mammalian cell.

Claim 209. (Previously presented) The isolated or purified cell of claim 201, wherein the donor substrate CMP-SA is CMP-Neu5Ac (cytidine monophosphate-*N*-acetylneuraminic acid).

Claim 210. (Previously presented) The isolated or purified cell of claim 201 wherein said CMP-sialic acid synthase gene and said sialic acid phosphate synthase gene are isolated from a human source.

Claim 211. (Previously presented) The cell of claim 201 wherein said cell is provided with ManNAc by addition of ManNAc to media in which said cell is grown.

Claim 212. (Previously presented) The cell of claim 201 wherein said cell is provided with ManNAc by genetically engineering said cell to catalyze synthesis of ManNAc.

Claim 213. (Previously presented) The cell of claim 212, wherein said cell is genetically engineered to contain or express an epimerase that synthesizes ManNAc.

Claim 214. (Previously presented) The cell of claim 213, wherein said epimerase is UDP-

GlcNAc-2 epimerase.

Claim 215. (Previously presented) The cell of claim 213, wherein said epimerase is GlcNAc-2 epimerase.

Claim 216. (Previously presented) The cell of claim 201 wherein said cell also contains SA precursor GlcNAc above endogenous levels as a result of supplementation of cell growth medium with SA precursor GlcNAc.

Claims 217-225. Cancelled

Claim 226. (Currently amended) An isolated or purified cell from a recombinant or genetically engineered cell line which contains and co-expresses

i) a ~~CMP~~ cytidine monophosphate-sialic acid (CMP-SA) synthase gene, wherein said CMP- SA-synthase gene encodes a polypeptide that catalyzes the formation of CMP-SA from SA and cytidine triphosphate (CTP), and wherein said (CMP-SA) synthase gene encodes polypeptide is

- a) ~~the~~ a polypeptide represented by SEQ ID NO:4 or
- b) a variant of the polypeptide represented by SEQ ID NO:4 that contains conservative amino acid substitutions and in which CMP-SA synthase structure and function are conserved; and

ii) a sialic acid phosphate synthase (SAS) gene, wherein said SAS gene encodes a polypeptide that catalyzes the formation of sialic acid phosphate from N-acetylmannosamine (ManNAc) and phosphoenolpyruvate (PEP), and said (SAS) gene encoding: wherein polypeptide is

- a) [the] a polypeptide represented by SEQ ID NO: 6; or
- b) a variant of the polypeptide represented by SEQ ID NO:6 that contains conservative amino acid substitutions and in which SAS structure and function are

conserved; or

c) [the] a polypeptide represented by SEQ ID NO: 8; or

d) a variant of the polypeptide represented by SEQ ID NO: 8 that contains conservative amino acid substitutions and in which SAS structure and function are conserved,

~~to produce~~ said cell producing a donor substrate CMP-SA at a higher level than a cell from a parent cell line corresponding to said recombinant or genetically engineered cell line, wherein the donor substrate CMP-SA is CMP-KDN (cytidine monophosphate-2-keto-3-deoxy-D-glycero-D-galacto-nonoic acid).

Claim 227. (Previously presented) The isolated or purified cell of claim 226, which is an insect cell.

Claim 228. (Previously presented) The insect cell of claim 227, wherein said insect cell is of a species selected from the group consisting of:

(a) *Spodoptera frugiperda*;

(b) *Trichoplusia ni*;

(c) *Estigmena acrea*; and,

(d) *Drosophila*.

Claim 229. (Previously presented) The isolated or purified cell of claim 226, which is a yeast cell.

Claim 230. (Previously presented) The isolated or purified cell of claim 226, which is a plant cell.

Claim 231. (Previously presented) The isolated or purified cell of claim 226, which is a bacterial cell.

Claim 232. (Previously presented) The isolated or purified cell of claim 226, which is a fungal

cell.

Claim 233. (Previously presented) The isolated or purified cell of claim 226, which is a mammalian cell.

Claim 234. (Previously presented) The isolated or purified cell of claim 226, wherein said CMP-sialic acid synthase gene and said sialic acid phosphate synthase gene are isolated from a human source.

Claim 235. (Currently amended) An isolated or purified cell from a recombinant or genetically engineered cell line which contains and co-expresses

i) a ~~CMP~~ cytidine monophosphate-sialic acid (CMP-SA) synthase gene, wherein said CMP-SA-synthase gene encodes a polypeptide that catalyzes the formation of CMP-SA from SA and cytidine triphosphate (CTP), and wherein said ~~CMP-sialic acid~~ SA synthase gene comprises

a) a nucleotide sequence represented by SEQ ID NO: 3, or

b) a polynucleotide that hybridizes to the nucleotide sequence represented by SEQ ID NO:3 under stringent conditions, and

ii) a sialic acid phosphate synthase gene wherein said SAS gene encodes a polypeptide that catalyzes the formation of sialic acid phosphate from N-acetylmannosamine (ManNAc) and phosphoenolpyruvate (PEP), and wherein said sialic acid phosphate synthase gene comprises

a) a nucleotide sequence represented by SEQ ID NO: 5, or

b) a polynucleotide that hybridizes to the nucleotide sequence represented by SEQ ID NO:5 under stringent conditions,

to produce said cell producing a donor substrate CMP-SA at a higher level than a cell from a parent cell line corresponding to said recombinant or genetically engineered cell line, wherein the donor substrate CMP-SA is CMP-KDN (cytidine monophosphate-2-keto-3-deoxy-D-glycero-D-galacto-nononic acid).



Claim 236. (Previously presented) The isolated or purified cell of claim 235, which is an insect cell.

Claim 237. (Previously presented) The insect cell of claim 236, wherein said insect cell is of a species selected from the group consisting of:

- (a) *Spodoptera frugiperda*;
- (b) *Trichoplusia ni*;
- (c) *Estigmena acrea*; and,
- (d) *Drosophila*.

Claim 238. (Previously presented) The isolated or purified cell of claim 235, which is a yeast cell.

Claim 239. (Previously presented) The isolated or purified cell of claim 235, which is a plant cell.

Claim 240. (Previously presented) The isolated or purified cell of claim 235, which is a bacterial cell.

Claim 241. (Previously presented) The isolated or purified cell of claim 235, which is a fungal cell.

Claim 242. (Previously presented) The isolated or purified cell of claim 235, which is a mammalian cell.

Claim 243. (Previously presented) The isolated or purified cell of claim 235, wherein said CMP-sialic acid synthase gene and said sialic acid phosphate synthase gene are isolated from a human source.